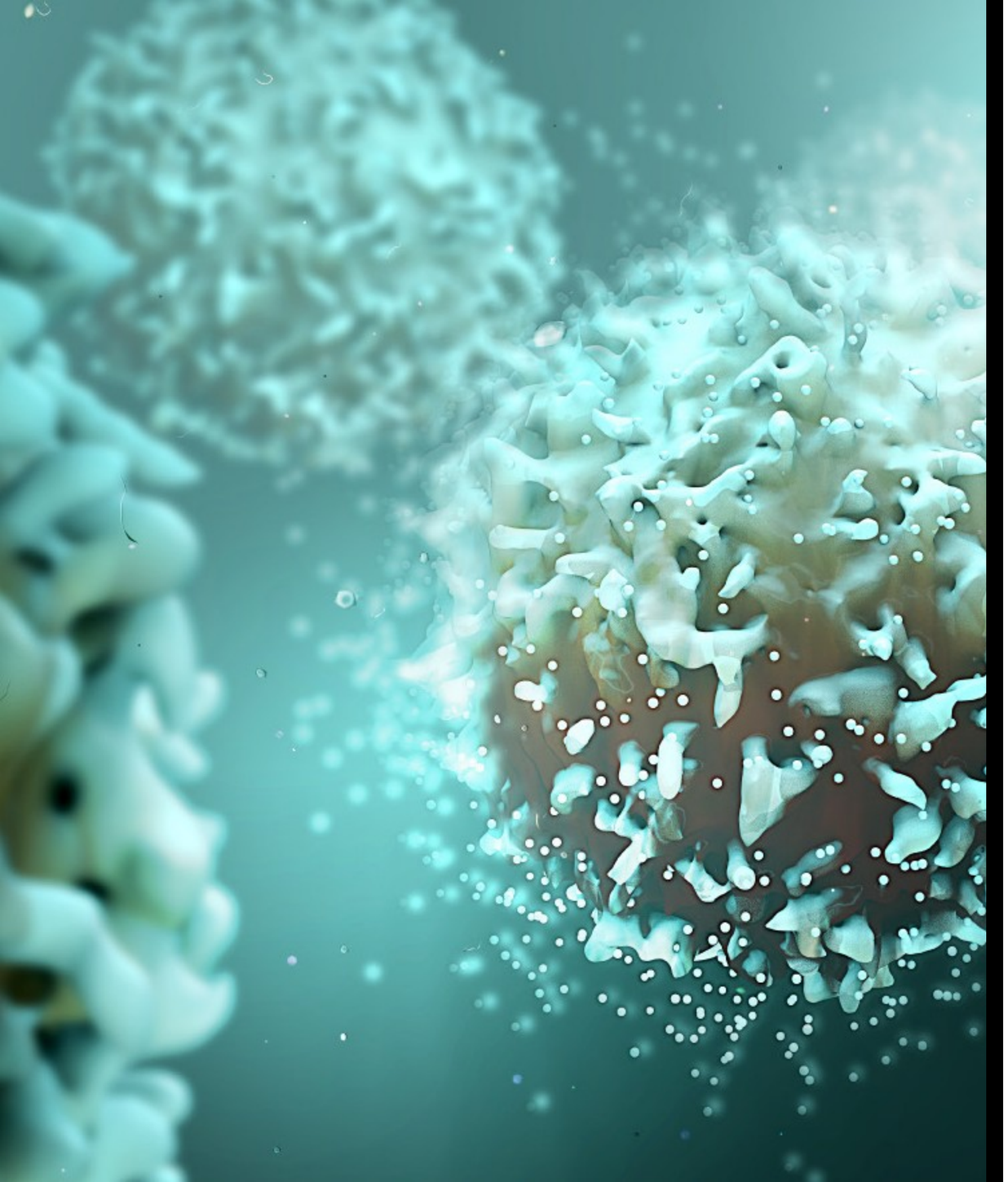
HESI IMMUNO-SAFETY TECHNICAL COMMITTEE

On-demand Training Course

Gene Therapy

Kathleen Meyer, MPH, PhD, DABT from Sangamo Therapeutics



Learning Objectives

- Become familiar with the field of gene therapy
- Gain understanding of the use recombinant AAV and lentiviral vectors in gene therapy
- Introduction to genome editing and gene regulation technologies
- Understand the considerations for designing nonclinical programs
- Nonclinical safety evaluation strategies for gene therapy investigational products

Agenda

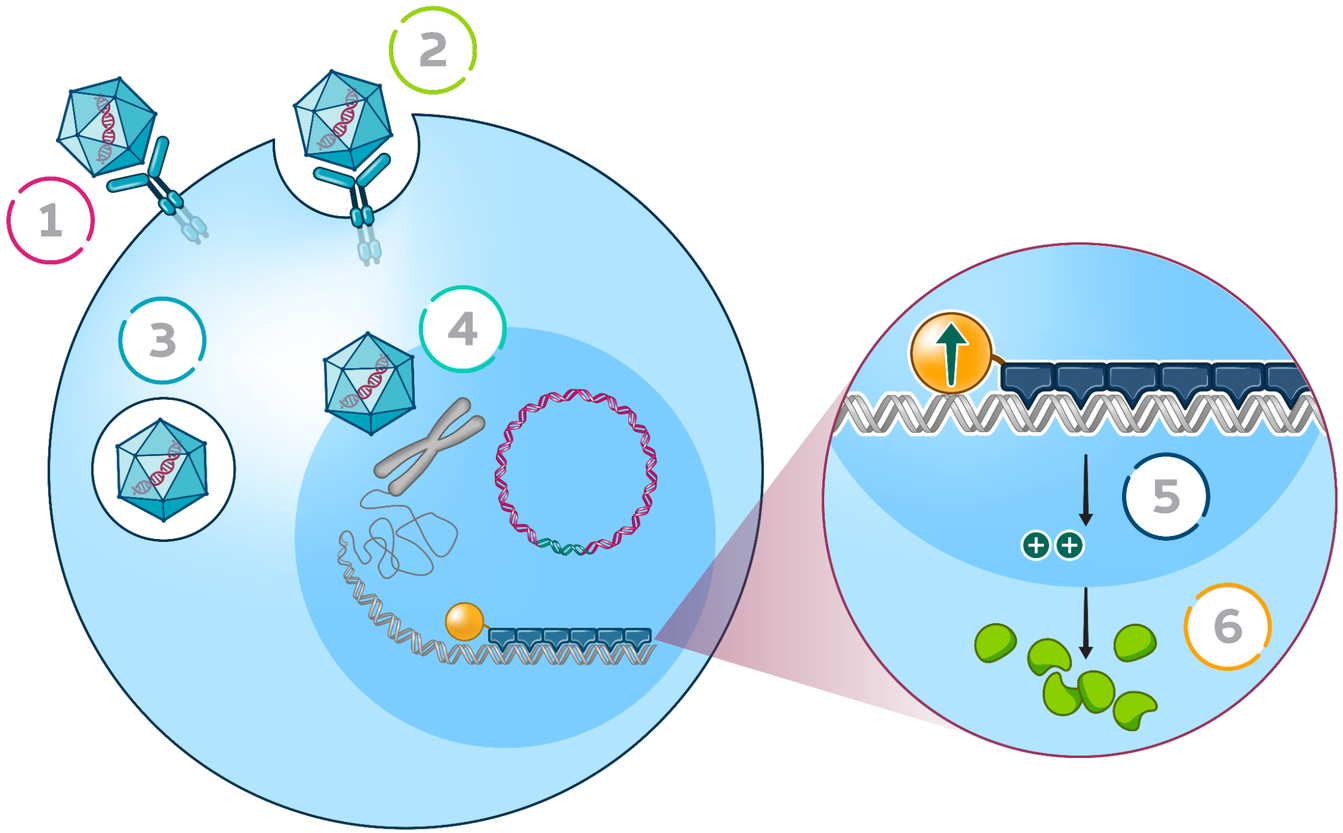
HESI IMMUNOSAFETY TECHNICAL COMMITTEE

1. Introduction to AAV Gene Therapy (GT)
2. Gene Therapy Delivery
3. Designing the Nonclinical Program
4. Nonclinical Safety Evaluation Strategies
5. Summary & Conclusions

Introduction to Gene Therapy

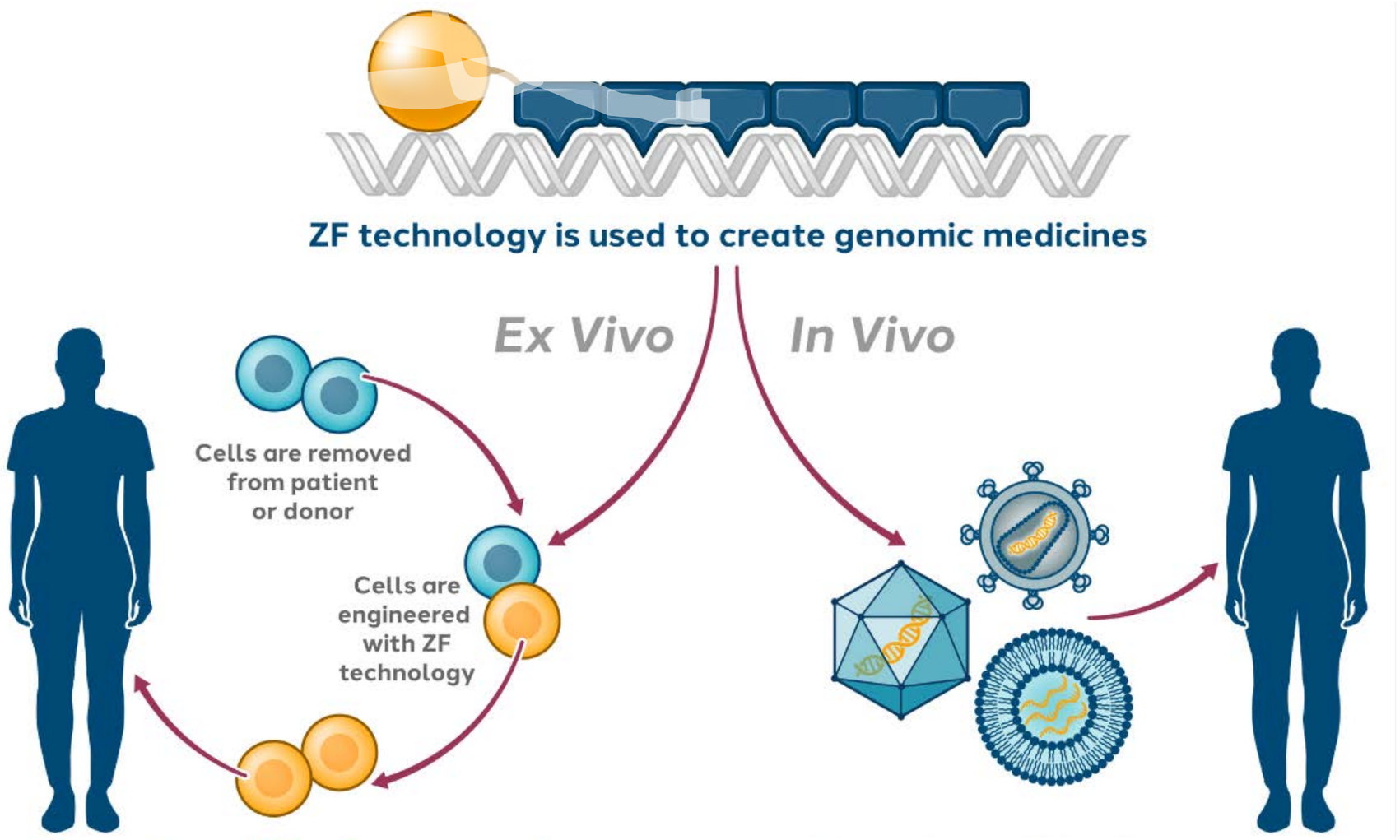
What is gene therapy?

Gene therapy (GT) is the introduction, removal or change in genetic material to treat human disease



1. Recombinant adeno-associated virus (AAV) vector with transgene binds to cell receptor
2. AAV internalization and uptake into cell
3. AAV trafficking into cytoplasm
4. AAV trafficking from cytoplasm into nucleus and expression of transgene
5. Specific and selective DNA binding and gene activation (i.e., zinc finger [ZF] transcription regulator)
6. Targeted increase (or decrease) in protein levels

Ex vivo or in vivo GT strategy?



Genomic medicine is composed of nuclease engineered or lentivirus transduced cells

Genomic medicine is composed of GT technology packaged in vectors

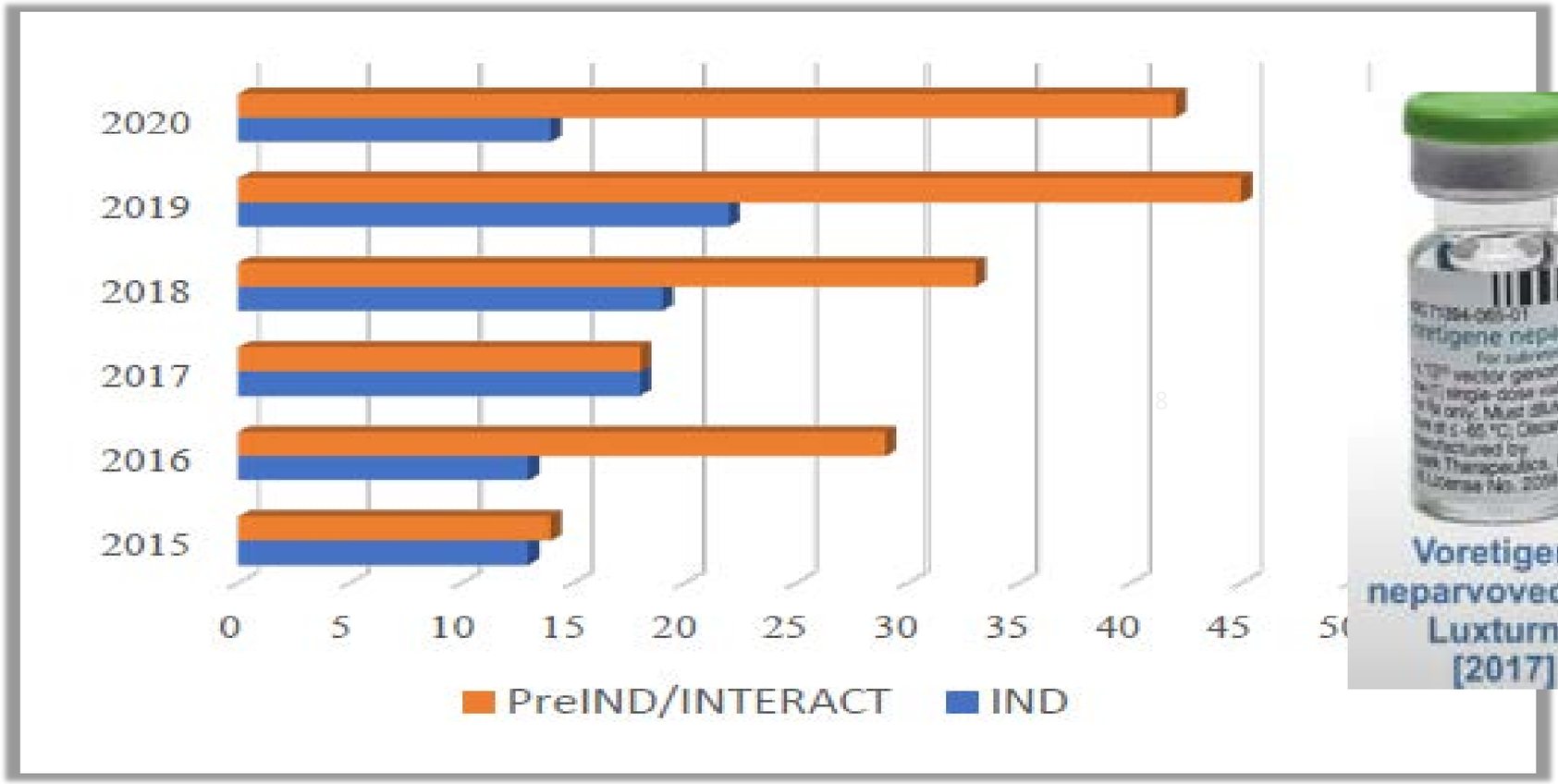
FDA CBER approved GT (single dose)

AAV CAR T HSPC Virus

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Product Name (Company)	Year	Generic Name	Description	Indication
HEMGENIX (Uniqure/CSLB)	2022	Etranacogene dezaparvovec-drib	AAV5-based therapy coding for Padua variant of Factor IX, under control of liver-specific promoter	Adult patients with Hemophilia B
ZYNTEGLO (Bluebird bio)	2022	betibeglogene autotemcel	Autologous HSC-based gene therapy transduced with LVV encoding $\beta^{A\text{-}T87Q}$-globin	Adult and pediatric patients with b-thalassemia who require regular RBC transfusions
SYSONA (Bluebird bio)	2022	elivaldogene autotemcel	Autologous HSC-based gene therapy transduced with LVV carrying ABCD1 cDNA that encodes normal ALDP	Slow progression of neurologic dysfunction in boys 4-17 years old with active cerebral adrenoleukodystrophy (CALD)
CARVYKTI (Janssen)	2022	ciltacabtagene autoleucel	BCMA-directed genetically modified autologous T cell immunotherapy	Adult patients with relapsed or refractory (r/r) multiple myeloma after four or more lines of therapy
ABECMA (Celgene; BMS)	2021	idecabtagene vicleucel	BCMA-directed genetically modified autologous CAR T cell immunotherapy	Adult patients with r/r multiple myeloma after four or more lines of therapy
BREYANZI (Juno/BMS)	2021	lisocabtagene maraleucel	CD19-directed genetically modified autologous CAR T cell immunotherapy	Adult patients with r/r large B-cell lymphoma not otherwise specified
TECARTUS (Kite/Gilead)	2020	brexucabtagene autoleucel	CD19-directed genetically modified autologous CAR T cell immunotherapy	Treatment of adult patients with r/r mantle cell lymphoma
ZOLGENSMA (AveXis)	2019	onasemnogene abeparvovec-xioi	AAV9-based therapy coding for the *SMN1* gene under control of ubiquitous promoter	Treatment of pediatric patients less than 2 years of age with SMA with biallelic mutations in the survival motor neuron (SMN1) gene
KYMRIAH (Novartis)	2017	tisagenlecleucel	CD19-directed genetically modified autologous CAR T cell immunotherapy	Patients up to 25 yrs with B-cell precursors ALL that is refractory or in second or later relapse. Adult patients with r/r large B-cell lymphoma after 2+ lines of systemic therapy
LUXTERNA (Spark)	2017	voretigene neparvovec-rzyl	AAV2-based therapy coding for the *RPE65* gene, under control of chicken β actin promoter and CMV enhancer	Treatment of patients with confirmed biallelic *RPE65* mutation-associated retinal dystrophy.
YESCARTA (Kite/Gilead)	2017	axicabtagene ciloleucel	CD19-directed genetically modified autologous CAR T cell immunotherapy	Treatment of adult patients with r/r large B-cell lymphoma after 2+ lines of systemic therapy. Adult patients with r/r follicular lymphoma after 2+ lines of systemic therapy
IMLYGIC (Amgen/BioVex)	2015	talimogene laherparepvec	Genetically modified HSV oncolytic viral therapy expressing GM-CSF	Local treatment of unresectable cutaneous, subcutaneous and nodal lesions in patients with melanoma recurrent after initial surgery

AAV GT meetings with OTAT 2015-2020
99 AAV GT INDs



FDA Cellular, Tissue and Gene Therapy Advisory Committee, Sep 2-3, 2021

FDA CDER approved ASOs and siRNAs (multi dose)

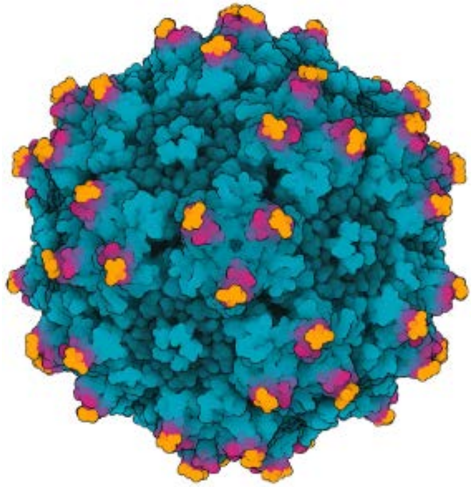
Product Name (Company)	Year	Generic Name	Description	Indication
QALSODY (Biogen)	2023	Tofersen	**ASO (antisense oligonucleotide)** targeting SOD1 mRNA	Amotrophic lateral sclerosis
AMONDYS 45 (Sarepta)	2021	Casimersen	**ASO** targeting exon 45 of dystrophin pre-mRNA; exon skipping	Duchenne muscular dystrophy
LEQVIO TM (Novartis)	2021	Inclisiran	siRNA (small interfering RNA) targeting PCSK9 mRNA	Hypercholesterolemia
OXLUMO (Alnylam)	2020	Lumasiran	siRNA targeting HAO1 mRNA	Primary hyperoxaoluria type 1 (PH1)
VILTEPSO (NS Pharma)	2020	Vitolarsen	**ASO** targeting exon 53 of dystrophin pre-mRNA; exon skipping	Duchenne muscular dystrophy
VYONDLYS (Sarepta)	2019	Golodirsen	**ASO** targeting exon 53 of dystrophin pre-mRNA; exon skipping	Duchenne muscular dystrophy
GIVLAARI (Alnylam)	2019	Givosiran	siRNA targeting aminolevulinate synthase 1 mRNA	Acute hepatic porphyrias
TEGSEDI (Akcea Ther)	2018	Inotersen	**ASO** targeting exon 53 of dystrophin pre-mRNA; exon skipping	Heredity transthyretin amyloidosis, polyneuropathy
ONPATTRO (Alnylam)	2018	Patisiran	siRNA targeting transthyretin mRNA	Adults with polyneuropathy of hereditary transthyretin-mediated amyloidosis
SPINRAZA (Biogen)	2016	Nusineren	**ASO** targeting SMN2 mRNA	Spinal muscular atrophy
EXONDLYS SI (Sarepta)	2016	Eleplirsen	**ASO** targeting exon 51 of dystrophin pre-mRNA; exon skipping	Duchenne muscular dystrophy
DEFITELIO (Jazz Pharma)	2016	Defibrotide	Mixture of ss-DNA and ds-DNA; In vitro, defibrotide sodium enhances the enzymatic activity of plasmin to hydrolyze fibrin clots	Hepatic veno-occlusive disease

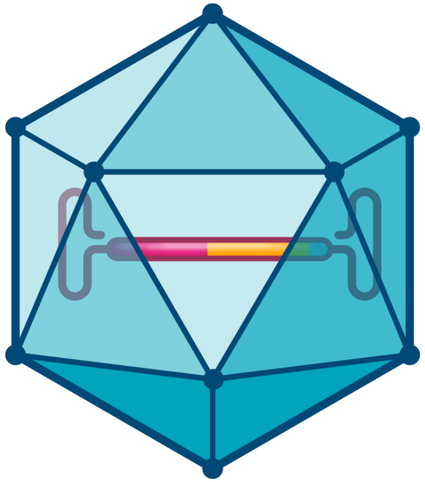
GT Delivery

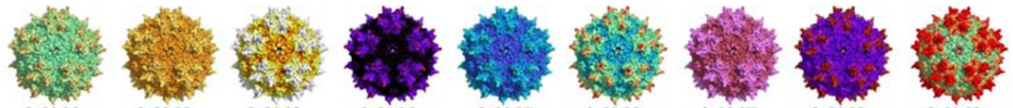
Adeno-associated viral (AAV) vectors

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

- Small single-strand DNA virus (20-25 nm diameter)
- 4.7 kb genome
- Widespread in animals and humans
- Non-pathogenic
- More than 100 serotypes with different tropism
- Only replicates in presence of helper virus (e.g., adenovirus, HSV-1, EBV)
- Can be engineered to express a therapeutic gene







	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Mouse	• Heart • Liver • Skeletal Muscle	• Heart • Liver • Muscle	• Heart • Liver	• Heart • Liver • Lung	• Liver	• Heart • Liver • Skeletal Muscle	• Liver • Skeletal Muscle	• Heart • Liver • Brain • Muscle	• Brain • Heart • Liver • Lung • Skeletal Muscle
Human	• CNS • Heat • Skeletal Muscle	• CNS • Eye		• Eye	• CNS	• CNS • Heart • Skeletal Muscle		• CNS • Eye	• CNS • Heart • Skeletal Muscle

AAV structure

- AAV capsid composed of 60 copies of total viral protein
- ITRs- Inverted Terminal Repeats of 145kb
- Required for viral replication and packaging
  - Rep - for viral replication
  - Cap - for viral capsid
  - VP1:VP2:VP3 in 1:1:10 ratio
  - Assembly activating protein (AAP) and other accessory protein (MAPP)
- Infect and persist in nondividing cells (episomal concatemers)
- For recombinant AAV, the rep and cap genes are removed, and therapeutic transgene sequences inserted

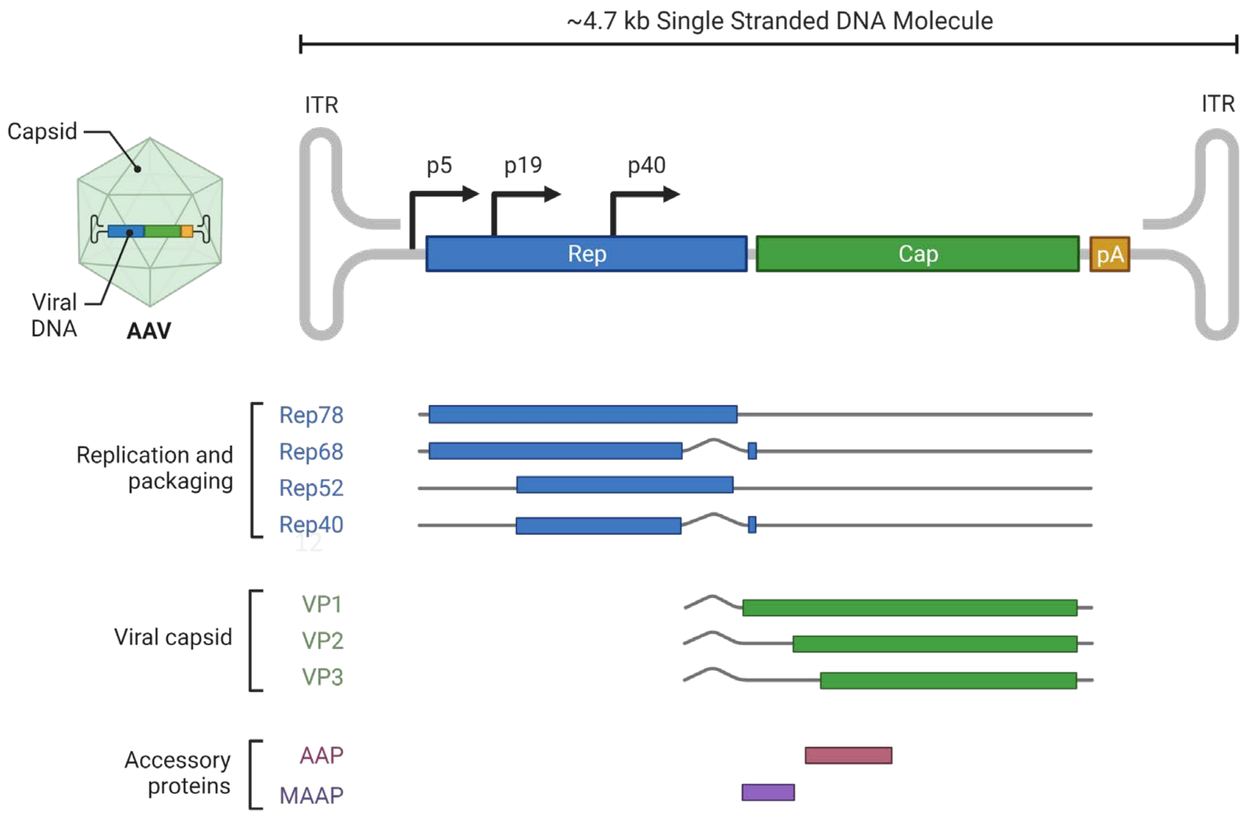
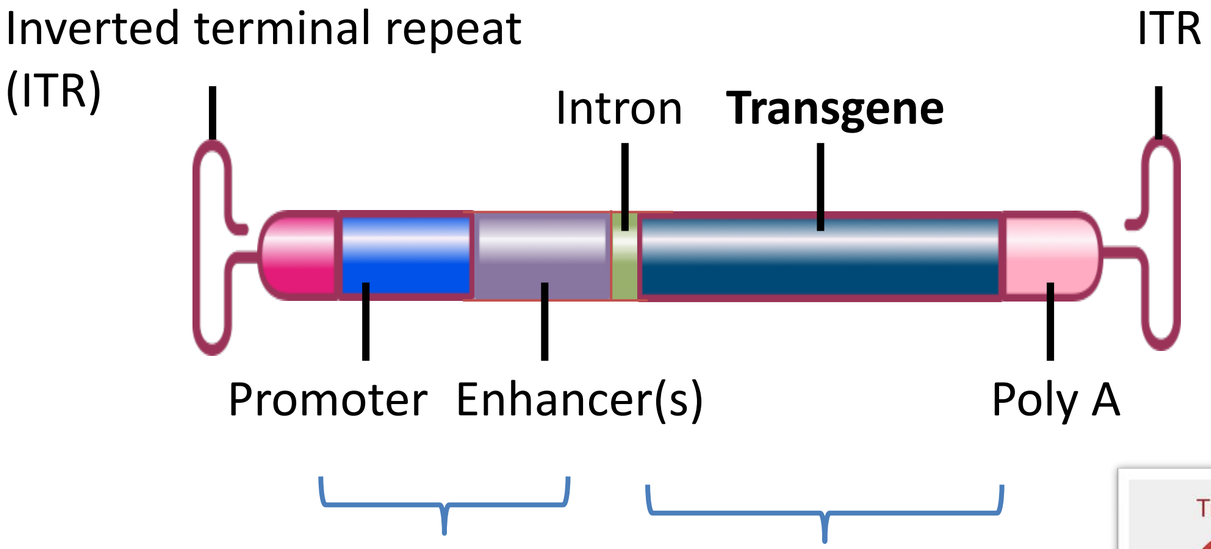


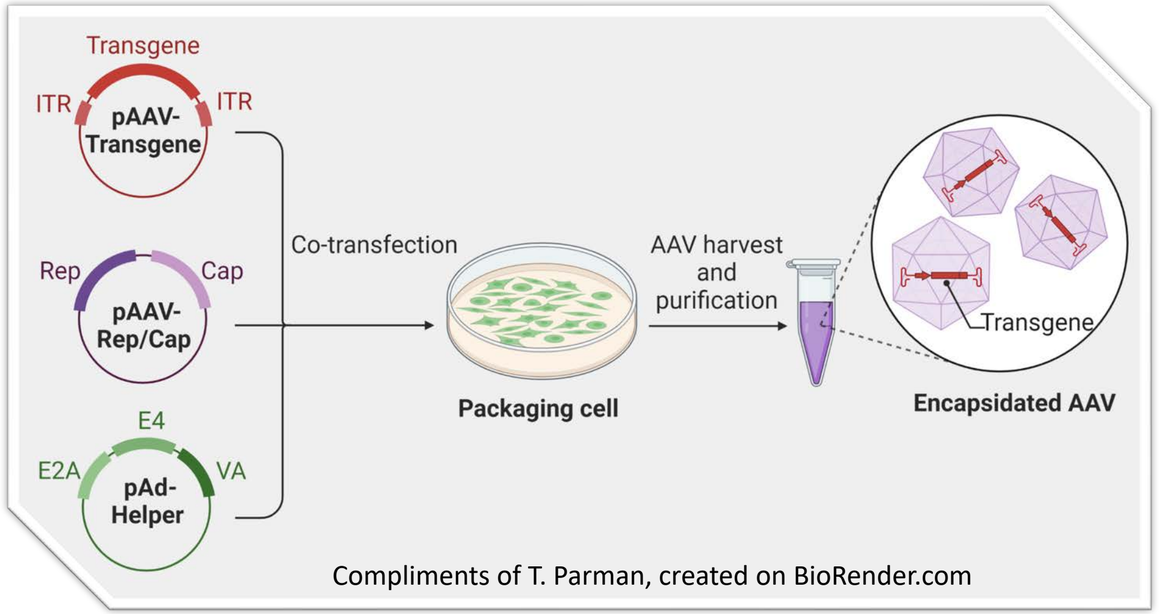
Figure for AAV Structure and production created by T. Parman using BioRender.com.

AAV construct design – art and science



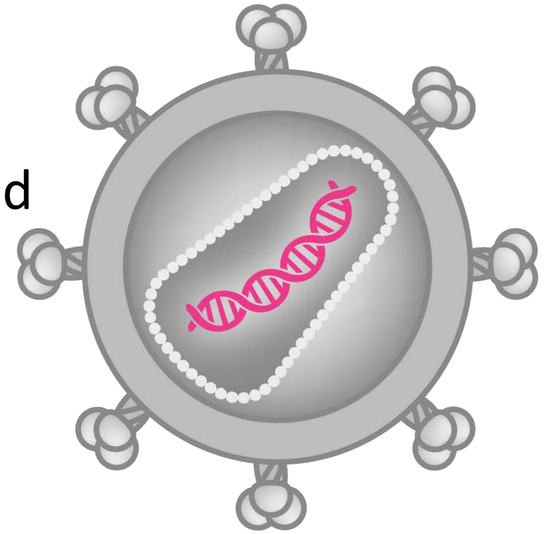
Ubiquitous or
tissue- specific

Gene size
Gene activity
Codon optimization
CpG content

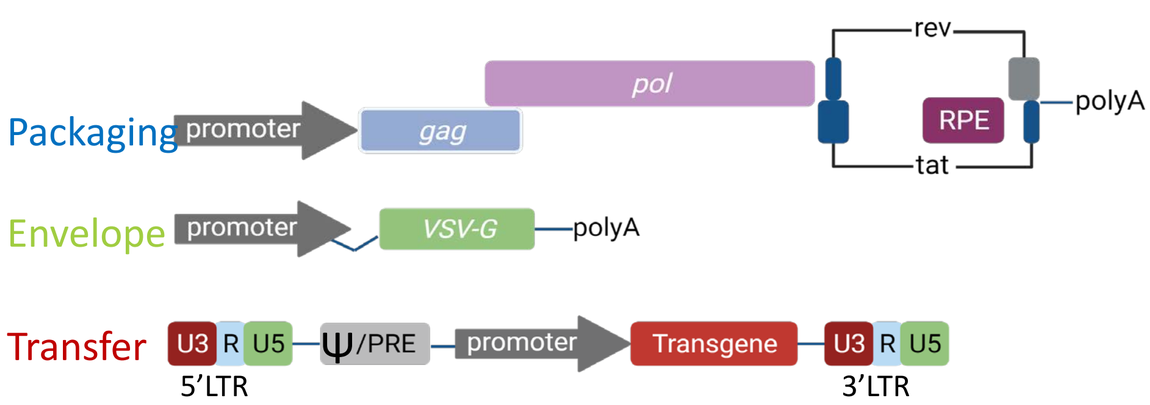


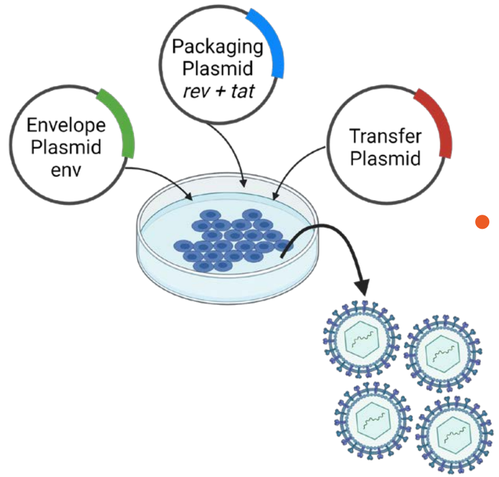
Lentiviral vectors (LVVs)

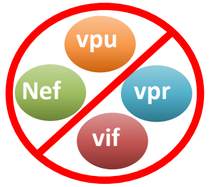
- Single-strand RNA virus (80-100 nm)
- 7-10 kb genome
- Transduces dividing and non-dividing cells
- Provides stable (long-term) and efficient expression of transgene
- Derived from HIV-virus, replication incompetent, and have evolved over the years (1st, 2nd and 3rd generation)
- Main risk is related to their unintended generation of replication-competent provirus, and non-random genome integration
- Mainly used in *ex vivo* cell modification:
  - To generate chimeric antigen receptor (CAR) T cells for cell therapy, and
  - For delivering genes into hematopoietic stem and progenitor cell (HSPC) therapy and CAR T cell therapy



2nd generation and self-inactivating (SIN) LVVs

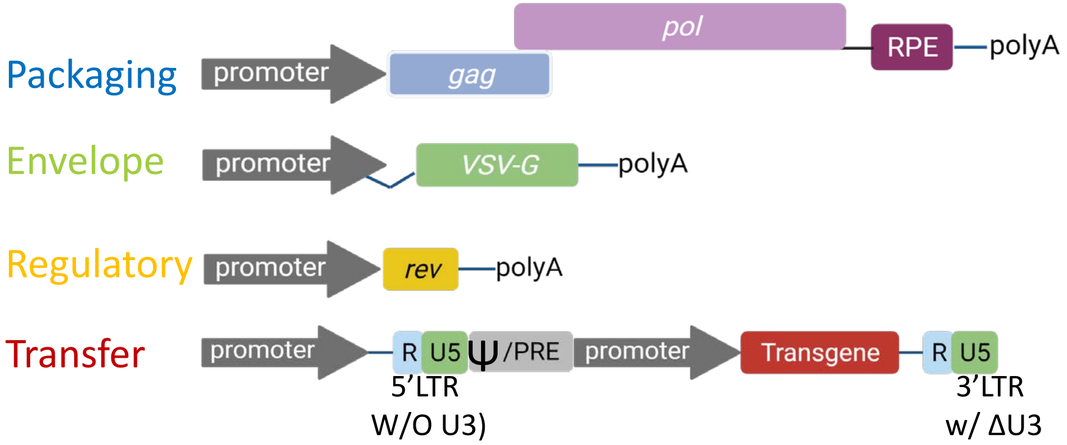


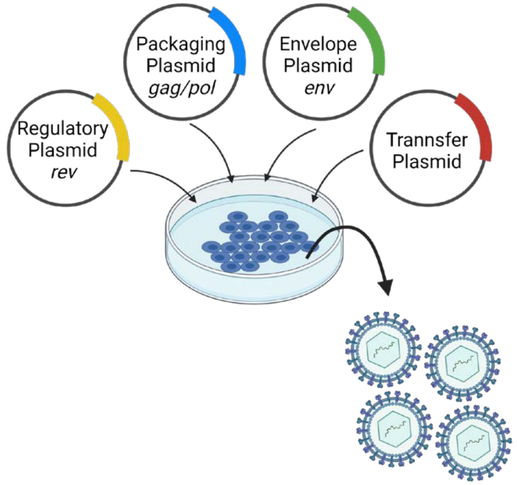




Genes required for viral propagation

- 2nd Generation
  - Low risk of integration
  - Can be generated in high titer





- 3rd Generation
  - Lower risk of integration
  - Reduces formation of RCLs
  - Titers can be lower than 2nd generation

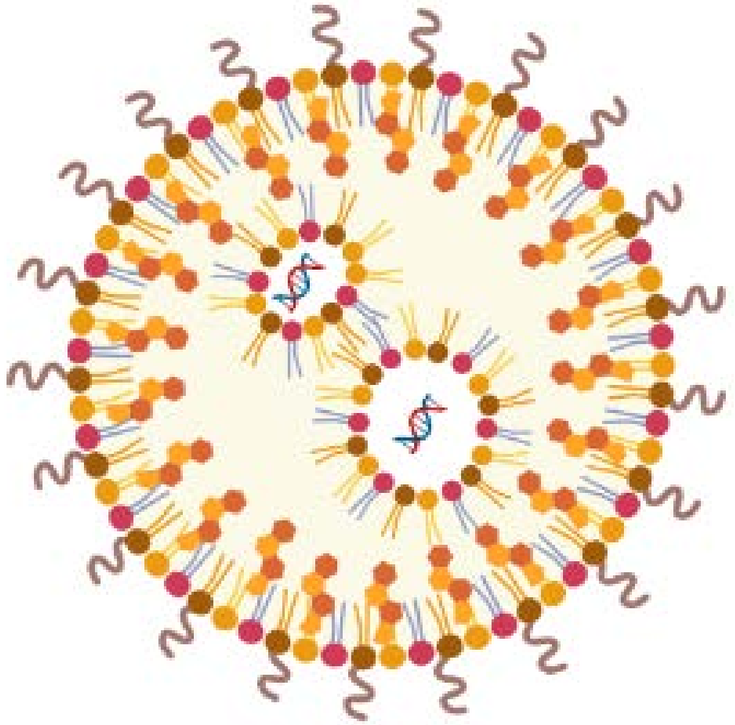
Figures Created by: T. Parman Using BioRender.com

Information from: Bulcha, Jt; Wang, Y; Ma, H et al (2021) Signal Transduction and Targeted Therapy 6: 53
Durand, S and Cimarelli, A (2011) Viruses 3:132-159
Gurumoorthy, N; Nordin, F; Tye, GJ et al (2022) Biomedicines 10(107): 1-19

RCL = Replication Competent LV

Lipid nanoparticles

- siRNA delivery
- Stable nucleic acid lipid particle (SNALP) technology
  - Onpattro® for transthyretin amyloidosis
  - First RNA/LNP to be evaluated in the clinic
- ~80 nm diameter
- Composition
  - Cationic lipid
  - Neutral helper lipid
  - Cholesterol
  - Diffusible polyethylene glycol (PEG; neutral charge at physiological pH)
- Liver and spleen targeting due to fenestrated epithelium (up to 100 nm diameter)
- Safety assessment of lipid components (small molecules) needed



GT Strategies

Gene therapy strategies

Gene Replacement

Cytoplasmic or secreted protein

Gene Silencing

Targeting mRNA for degradation or repression of translocation

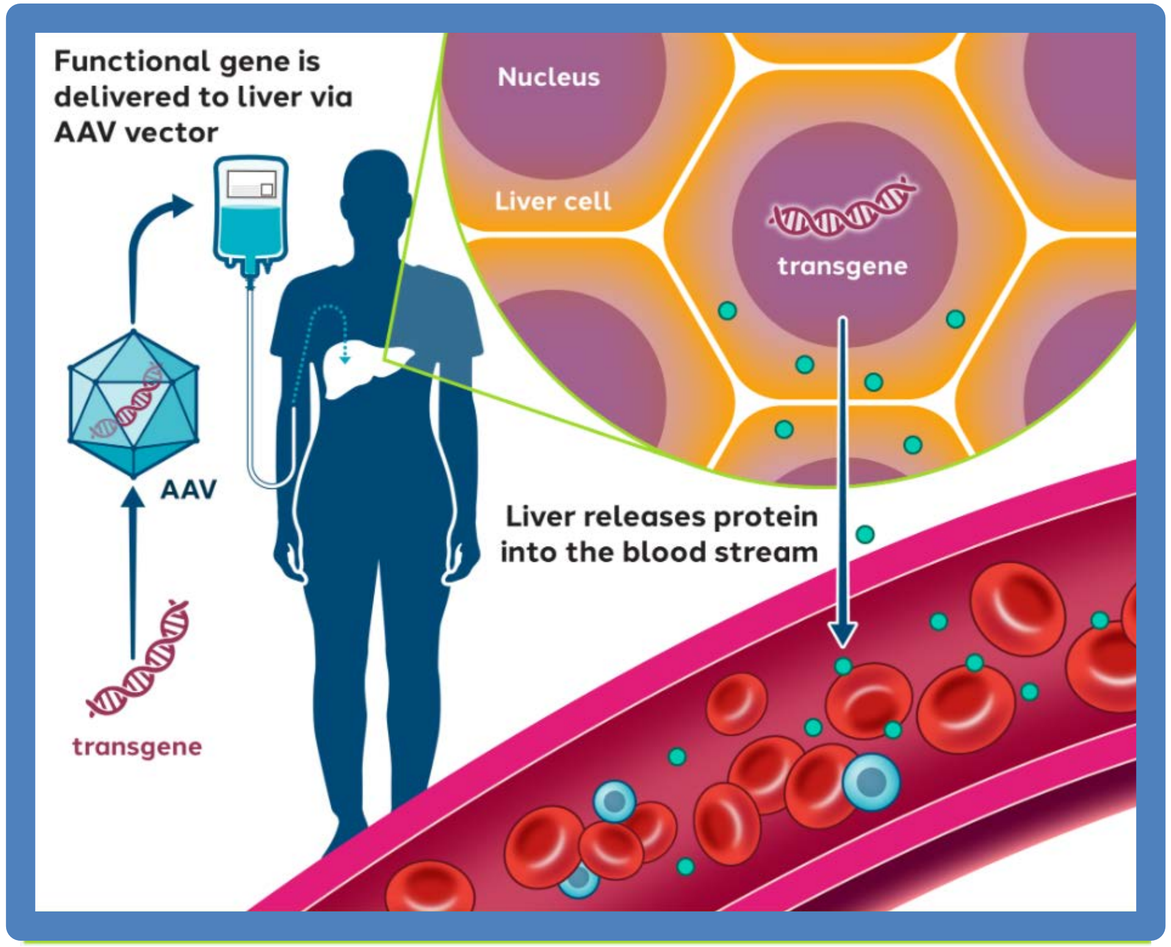
Genome Editing

Gene deletion, disruption or insertion

Gene Regulation

Gene repression or activation

Tissue-directed gene replacement



Marketed products

Targeted **AAV** GT

- Zolgensma – SMA
- Hemgenix – Hemophilia B

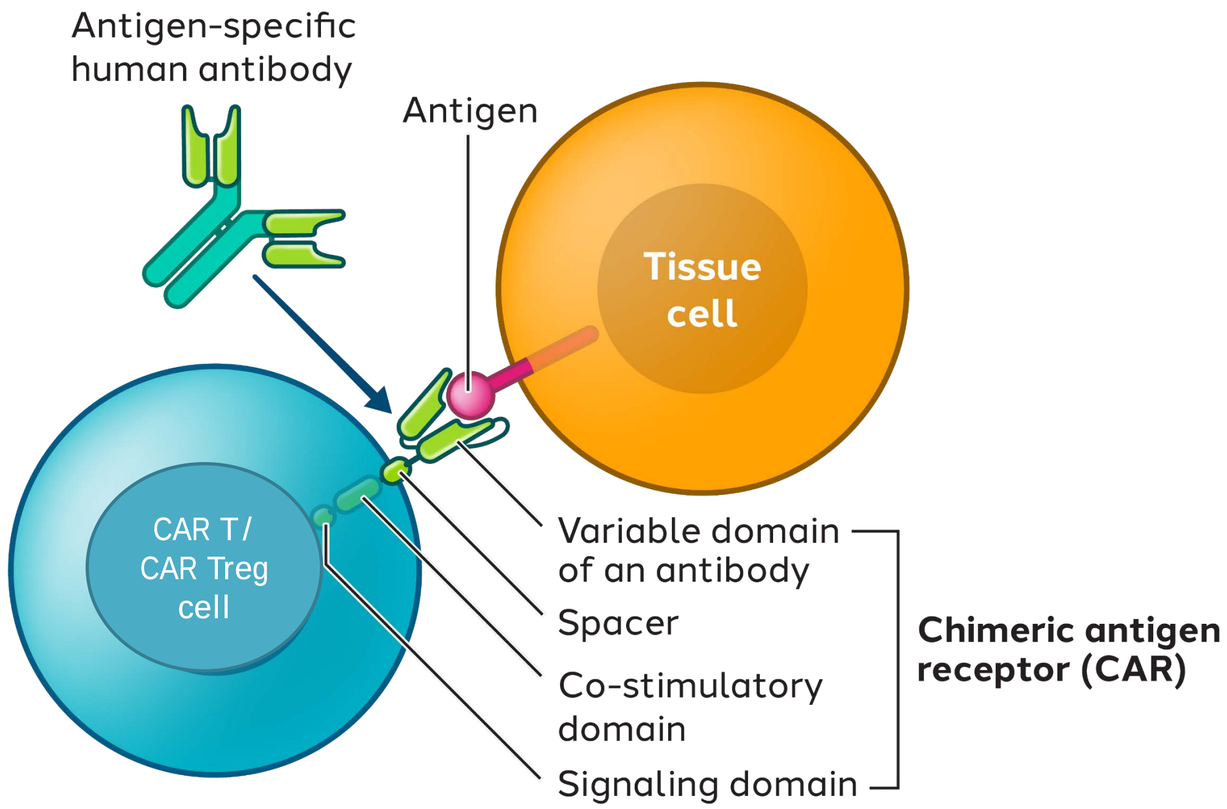
Ocular **AAV** GT

- Luxterna – RPE65

HSPC **LVV** GT

- Sysona – ABCD1
- Zynteglo - $\beta^{A\text{-}T87Q}$-globin

Chimeric antigen receptor (CAR) T cell therapy



LVV delivery of the CAR

Marketed products

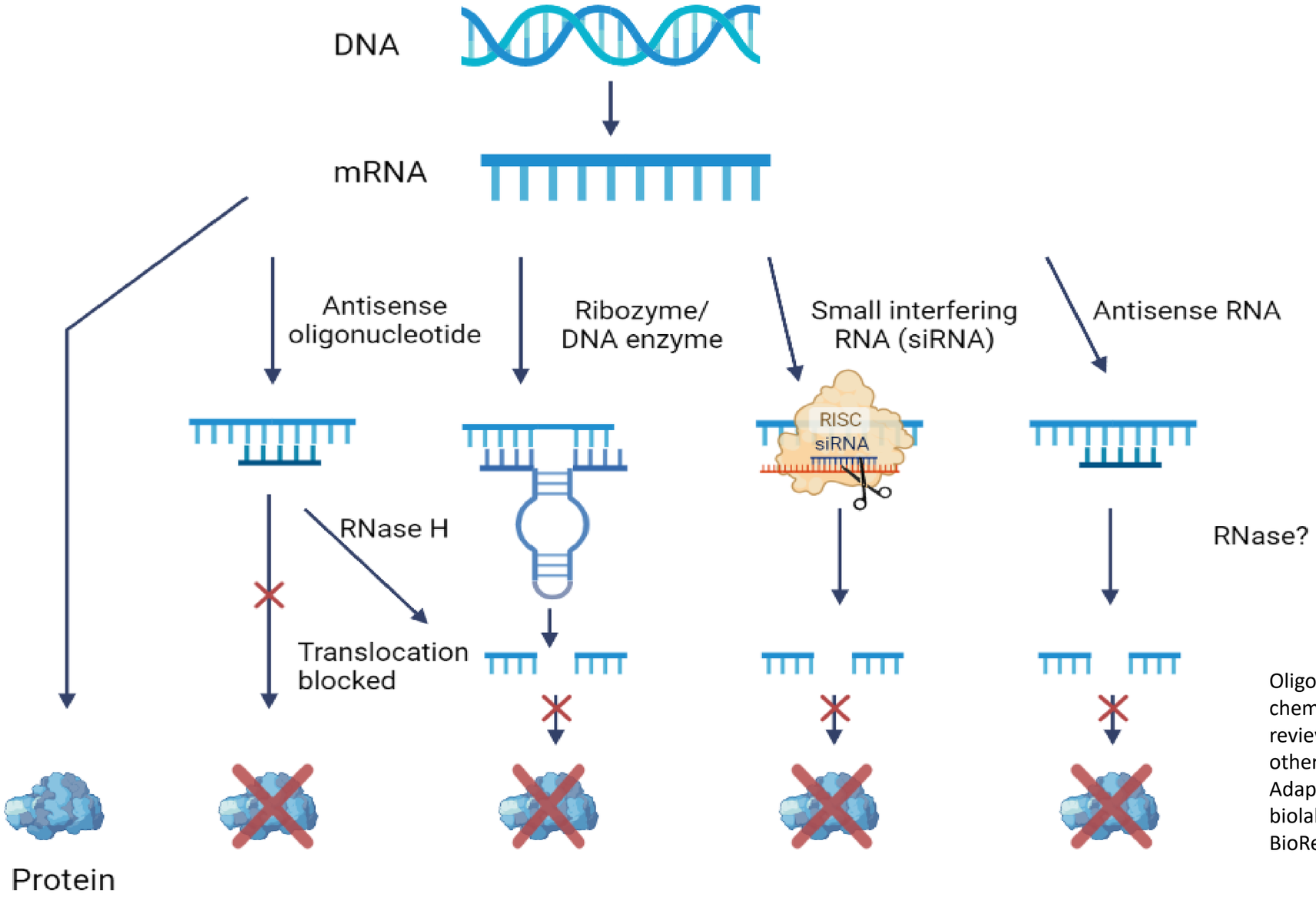
CAR T cells for oncology

- Kymriah, CD19 targeted
- Yescarta, CD19 targeted

CAR Treg cells clinical stage for mismatched kidney transplant

- TX200, HLA-A2 targeted

Gene silencing – siRNA and oligo strategies

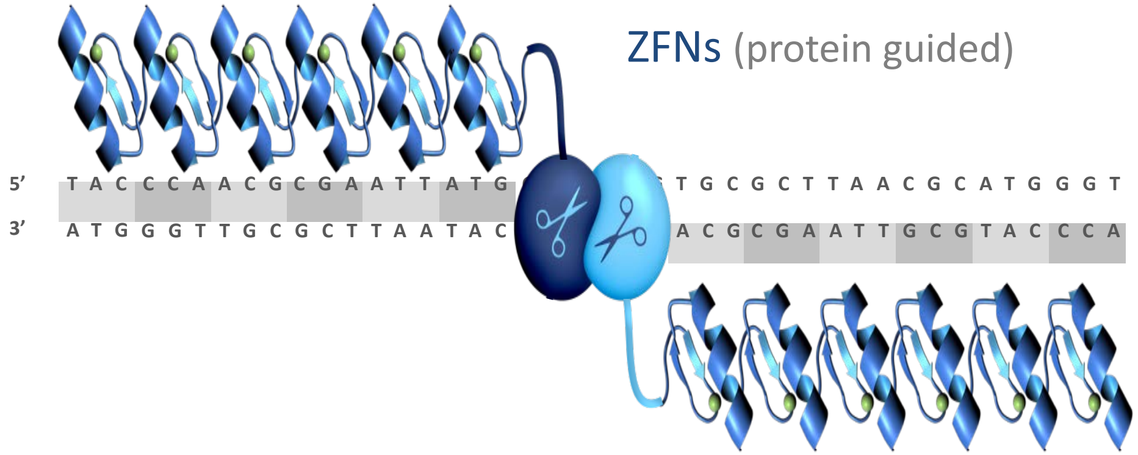


Oligonucleotides considered new chemical entities (NCE) and reviewed by CDER at US FDA; others by CBER
Adapted from Creative-biolabs.com using BioRender.com

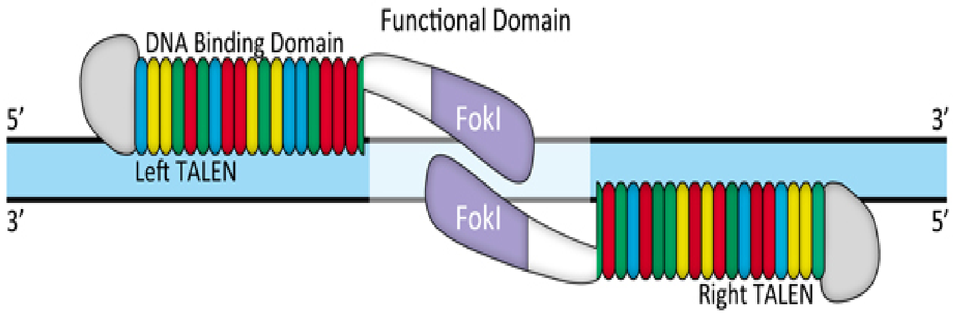
Genome editing (GE) – clinical stage engineered nucleases

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

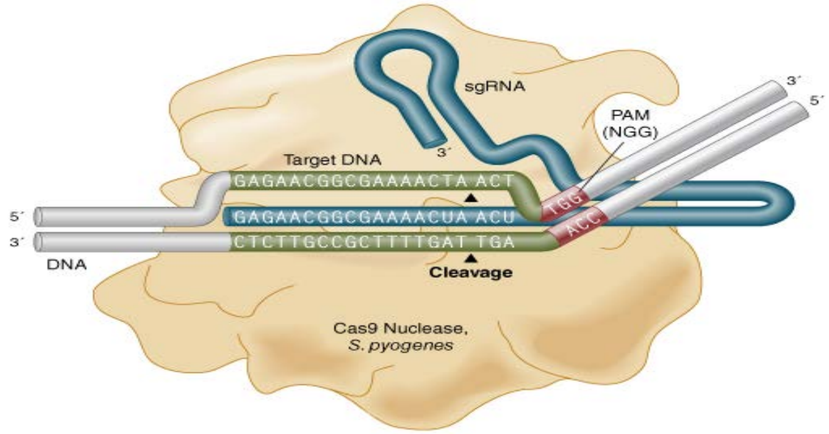
ZFNs (protein guided)



TALENs (protein guided)



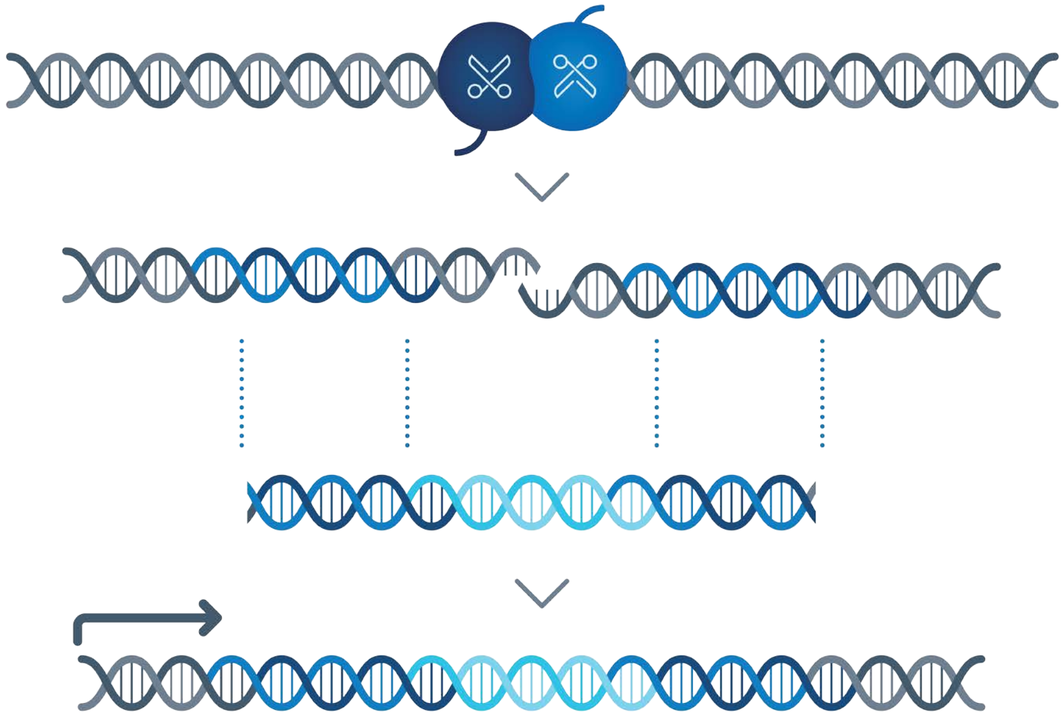
CRISPR-Cas9 (RNA guided)



Nuclease GE - DNA repair using non-homogenous end-joining (NHEJ)

Nuclease methods that induce DNA breaks are repaired by the same repair-mechanisms; no benefit of one type of nuclease modality over another with respect to downstream consequences of inducing DSBs (FDA Genome Editing Guidance 2022)

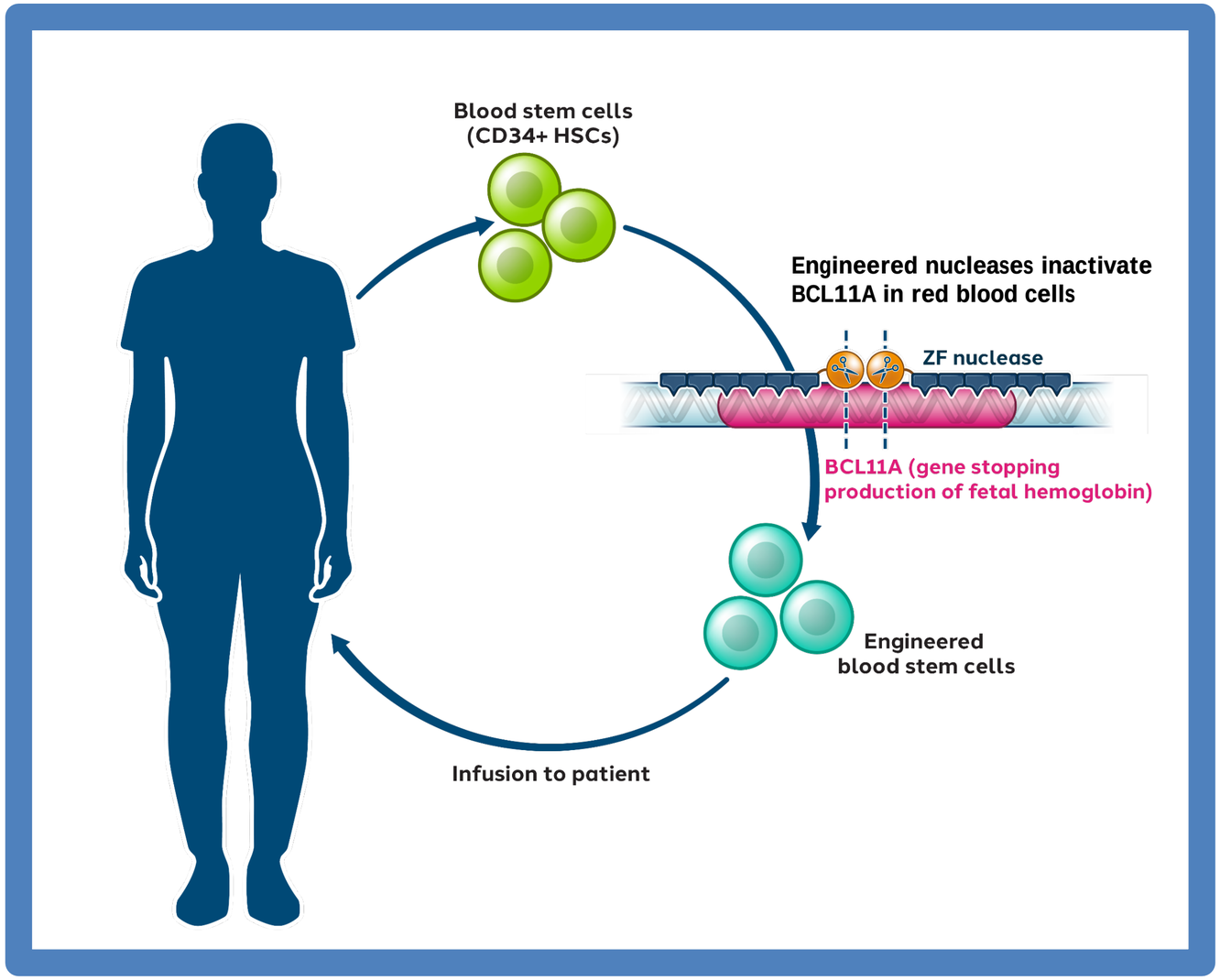
Nuclease GE - DNA repair using homology directed repair (HDR)



Human Therapeutic Transgene with Homology Arms

Insertion of Transgene into Specific Locus

Genome editing of hematopoietic stem & progenitor cells for sickle cell disease



Clinical Stage Programs with autologous CD34+ HSPCs

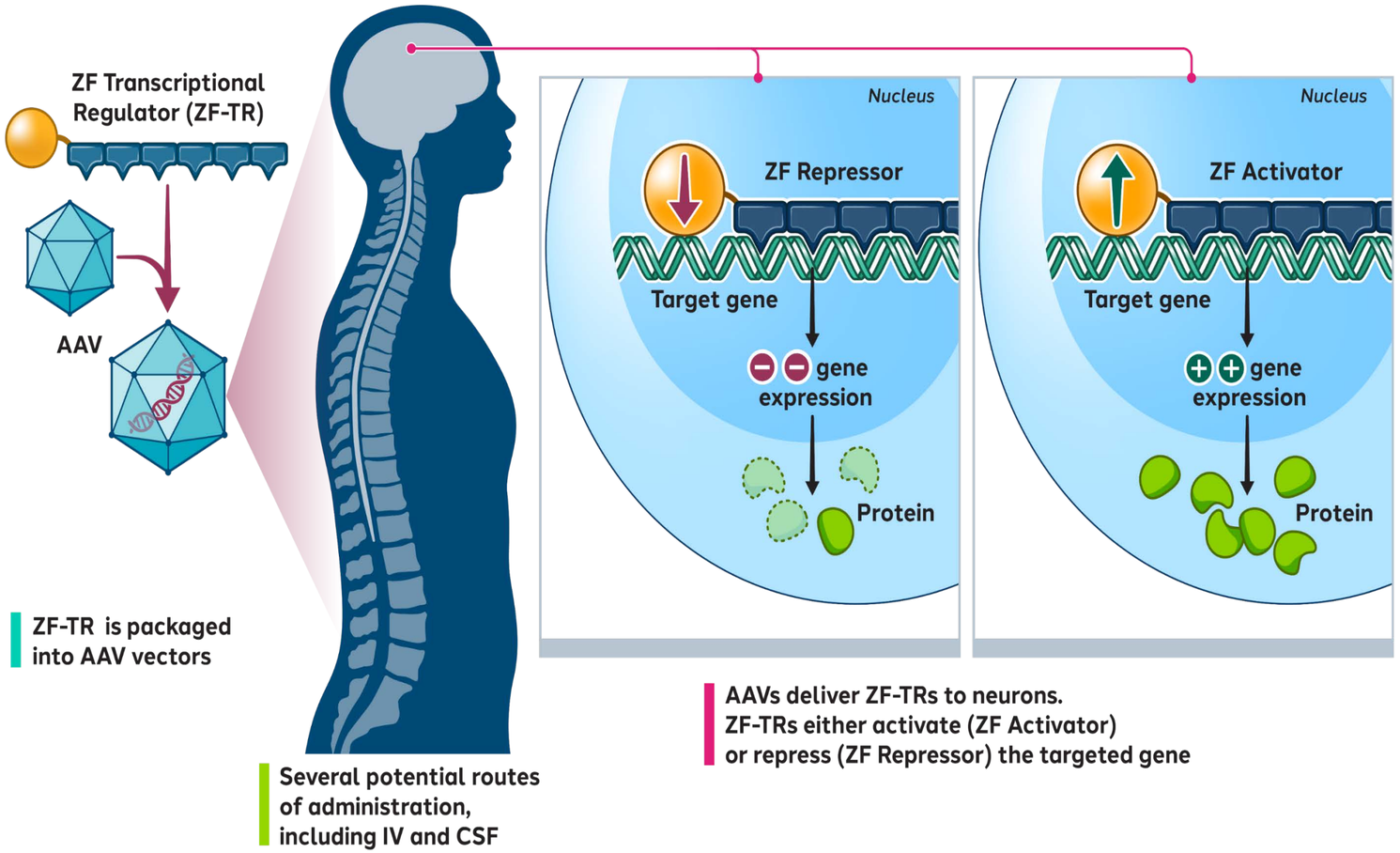
- Sangamo BIVV003: ZFNs targeting the erythroid BCL11A enhancer to increase HbF
- CRISPR/Vertex CTX001: CRISPR/Cas 9 targeting the erythroid DCL11A enhancer to increase HbF
- Editas EDIT-301: AsCas12 to edit promoter regions of gamma globin genes 1 and 2 to increase HbF
- Graphite Bio GPH101: HDR to replace mutated β globin gene with functional gene to restore HbA
- Beam Therapeutics BEAM-101: CRISPR-based base editing to correct mutated gene and restore HbA

ZF transcriptional regulators for CNS GT

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

ZF Transcriptional Regulators can be designed to:

- Reduce the expression of a pathogenic gene
- Selectively repress expression of a mutant allele while allowing for the expression of the healthy allele
- Activate the expression of genes that are inadequately expressed



The Nonclinical Program

Pharmacology, pharmacokinetics/biodistribution and toxicology assessment to support clinical evaluation

Target product profile (TPP)

Begin with the end in mind

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

The Clinical Plan starts with a TPP which includes:

- Mechanism of action of new GT drug
- The intended use of the new drug in patients
- Target indication
- Patient population to study
- Clinical strategy
- Single-dose but long-term durability
- Route of administration (RoA)
- Primary and secondary endpoints
- Biomarkers of exposure, effect and toxicity?

Nonclinical strategy to support the clinical plan

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

- Selection of animal species for therapeutic proof-of-concept studies and nonclinical safety evaluation?
- Strategy to assess the **pharmacodynamic activity**, **efficacy** , **PK/biodistribution** and **potential toxicity** of new gene therapy?
- Mirror intended clinical RoA
- Device/delivery
- Immunogenicity considerations
- Determine safety margin and clinical dosing plan
- Risk mitigation for treatment of patients
- Regulatory requirements

Regulatory guidance for gene therapy

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Long Term Follow-Up After Administration of Human Gene Therapy Products

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10
MD 20993-0002, or by calling
from the Internet at https://ww
regulatory-information-biologi

For questions on the content o
address listed above.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

12 November 2020
EMA/CAT/GTWP/671639/2008 Rev. 1 - corr
Committee for Advanced Therapies (CAT)

Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

Adoption by CAT	13 April 2012
Date for coming into effect	1 November 2012
Consultation with CAT, BWP (Rev.1)	14-16 March 2018
Draft adopted by CAT (Rev.1)	20 July 2018
Draft adopted by CHMP for release for consultation (Rev.1)	26 July 2018
Start of public consultation (Rev.1)	31 July 2018
End of consultation (deadline for comments) (Rev.1)	31 July 2019
Agreed by BWP	9 September 2020
Adopted by CAT	9 October 2020
Adopted by CHMP	12 November 2020
Date of coming into effect	1 June 2021
Keywords	***Genetically modified cell, advanced therapy, gene therapy, cell therapy, somatic cell, quality, non-clinical, clinical***

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact Telephone +31 (0)88 781 6000
An agency of the European Union
© European Medicines Agency, 2021. Reproduction is authorised provided the source is acknowledged.

Guidance for Industry

Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events

Additional copies of this guidanc
Manufacturers Assistance (HFM-
1448, or by calling 1-800-835-47
http://www.fda.gov/cber/guidelin

For questions on the content of th
Therapies at 301-827-5102.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 June 2010
EMEA/CHMP/GTWP/587488/2007 Rev. 1
Committee for the Medicinal Products for Human Use (CHMP)

Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors

Draft Agreed by BWP/SWP/EWP/PhVWP/VWP	December 2008 - January 2009
Draft Agreed by GTWP	January 2009
Draft Agreed by CAT	February 2009
Adoption by CHMP for release for consultation	19 March 2009
End of consultation (deadline for comments)	30 September 2009
Agreed by GTWP/BWP	March-May 2010
Adoption by CAT	June 2010
Adoption by CHMP	24 June 2010
Keywords	***Adeno-associated virus, self complementary adeno-associated virus, recombinant adeno-associated virus, production systems, quality, non-clinical, clinical, follow-up, tissue tropism, germ-line transmission, environmental risk, immunogenicity, biodistribution, shedding, animal models, persistence, reactivation, advanced therapy medicinal product, gene therapy medicinal product***

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 Facsimile +44 (0)20 7418 8416
E-mail info@ema.europa.eu Website www.ema.europa.eu
An agency of the European Union
© European Medicines Agency, 2010. Reproduction is authorised provided the source is acknowledged.

emea European Medicines Agency

London 30 May 2008
EMEA/CHMP/GTWP/125459/2006

COMMITTEE FOR THE MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON THE NON-CLINICAL STUDIES REQUIRED BEFORE FIRST CLINICAL USE OF GENE THERAPY MEDICINAL PRODUCTS

DRAFT AGREED BY GENE THERAPY WORKING PARTY	February 2007
DRAFT AGREED BY SAFETY WORKING PARTY	February 2007
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	March 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	September 2007
AGREED BY GENE THERAPY WORKING PARTY	April 2008
AGREED BY SAFETY WORKING PARTY	
ADOPTION BY CHMP	
DATE FOR COMING INTO EFFECT	
KEYWORDS	gene therapy medicinal products, non c

7 Westferry Circus, Canary Wharf, Lor
Tel. (44-20) 74 18 84 00 Fax (44-2
E-mail: mail@emea.europa.eu http://
© European Medicines Agency, 2008. Reproduction is author

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 March 2018
EMA/CAT/80183/2014
Committee for Advanced Therapies (CAT)

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products

Draft agreed by CAT drafting group	April 2014
Draft agreed by BWP and SWP	May 2014
Draft agreed by guideline consistency group	February 2015
Adoption by CAT	February 2015
Adoption by CHMP for release for consultation	March 2015
	31 August 2015
	July 2017
	July 2017
	October 2017
	February 2018
	February 2018
	March 2018

...ance on quality, non-clinical and clinical aspects of gene ...88/99)

...icinal products, advanced therapy medicinal products, ...clinical.

...ed Kingdom
...999 3555
...ffect

...duction is authorised provided the source is acknowledged.
An agency of the European Union

Guidance for Industry

Preclinical Assessment of Investigational Cellular and Gene Therapy Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or e-mail ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or e-mail address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2013

Species selection is critical for success

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Demonstrated biological response to product similar as expected for humans to generate data to guide clinical trial design

- Comparability of physiology and anatomy to humans
- Feasibility of using planned clinical delivery system or procedure

- Permissiveness/ susceptibility of infect- ion (and replication)
- Immune tolerance to human transgene expressed by GT product

- Justify selection and rationale in IND – assessment of relevance of each animal species

Establishing pharmacodynamic proof-of-concept

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Establish "**reason to believe**" for use of product in targeted population for clinical trial. Inform benefit side of risk/benefit assessment. Can contribute to animal species selection

- GT should be biologically active in model
- Pharmacology effective dose range (MED and optimal biologic dose)
- Optimize RoA and confirmation that product reaches target
- Optimization of dosing schedule
- Characterization of putative mechanism of action (MoA) or hypothesized biological activities of product
- In vitro assays to assess aspect of biology activity of investigational GCT product
- Use of model allows characterization of resulting morphological changes in conjunction with observable functional/behavioral changes

Animal model(s) for nonclinical assessment

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

- GT should be biologically active in model
- Healthy animals represent standard model test system to conduct traditional studies
- Hybrid pharmacology/toxicology studies in disease models can incorporate important safety parameters
  - ***Often recommended for gene therapy/genome editing programs***
  - Similarities/differences between pathophysiology of disease/injury model and humans
  - Effect of disease/injury state of animal on GT investigational product
  - Detrimental effects of product on existing disease/injury status
  - ***Gain understanding of how much transgene expression (or editing, gene regulation, etc.) is needed to impact disease state***
- Such data can supplement or possibly be used in lieu of toxicology studies in healthy animals

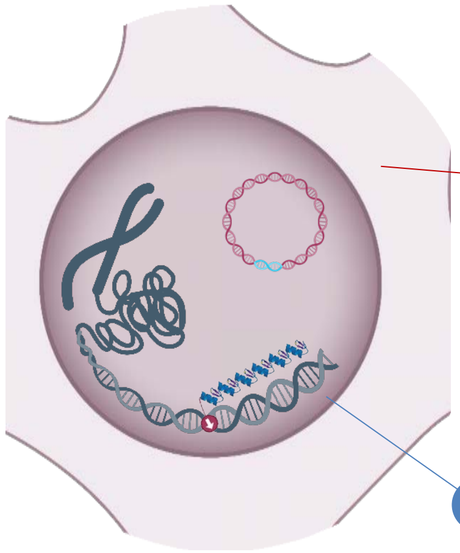
Animal models of disease/injury

- May provide insight into relationships of dose to pharmacodynamic activity (PD) and toxicity
  - How much transgene expression needed to impact disease?
  - How much editing is needed to result in PD effect?
  - How much gene repression needed to impact disease?
- May be preferable to healthy animals to assess activity and safety
- Opportunity for possible identification of activity /risk biomarkers for monitoring in clinical trials
- Limitations
  - Inherent variability of model
  - Limited historical/baseline data
  - Technical limitations with physiology, pathophysiology and anatomy constraints of model of disease/injury of interest
  - Animal care issues
  - Limited fidelity in modeling human

GT bioanalytical assays

- Vector titer (ddPCR assay)
- Dose formulation analysis (qPCR/ddPCR assay)
- Vector biodistribution and shedding (qPCR assay)
- Gene modification (editing) – insertions/deletions (Next gen sequencing)
- Transgene mRNA levels (RT-qPCR; in situ hybridization, single-cell analysis)
- Transgene protein levels (ELISA, activity assay, immunohistochemistry)
- Target engagement assessment (mRNA and/or protein)
- Pharmacodynamic activity (substrate and/or metabolite reduction; assays)
- Anti-AAV antibody assay (neutralizing and/or total antibody)
- Anti-expressed transgene antibody assay

Gene expression by RT-qPCR as target engagement and pharmacodynamic biomarkers



1. Expression of ZFP-TF transgene from non-integrating AAV episome

Absolute quantitation of transgene mRNA copy number per cell in treated samples

2. Durable change of target gene expression

Fold change of target gene expression between treated and untreated

The correlation between the two readouts is essential in demonstrating target engagement to support pharmacology and IND-enabling studies for genome regulation modalities. **How much is enough to impact disease**?

Nonclinical safety assessment

- Typically, two species; justification possible for one species
- Safety assessment and acceptable risk-benefit ratio for proposed trial
  - Identification, characterization, and quantification of potential local and systemic toxicities
  - Onset (acute or delayed), possibility for resolution of any toxicities, and effect of product dose level on toxicology findings
- Considerations in design
  - Proposed clinical indication
  - Amount and quality of published preclinical or clinical safety information
  - Biological responsiveness of animal species to product, putative MOA, intrinsic properties of product
  - Pathophysiology of animal disease/injury model
  - Device experience

Key safety assessments for GT products (1)

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

AAV and LVV Vectors

- Biodistribution and level of persistence in target and non-target tissues
- Unique issues related to vector class (e.g., replication, shedding)
- Risk of DNA integration; insertional mutagenesis and germline transmission

Transgene Activity

- Levels of transgene expression
- Biologic response of the transgene (target expression/ functional consequences)
- Possible toxicity due to aberrant or excessive expression
- Risk of expression in non-target tissues

Kinetics and Risks of Expression

- AAV PK in serum
- Viral vectors, LNP components and transgene
- Local vs systemic /acute vs chronic / level vs duration expression
- Immunogenicity

Key safety assessments for GT products (2)

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

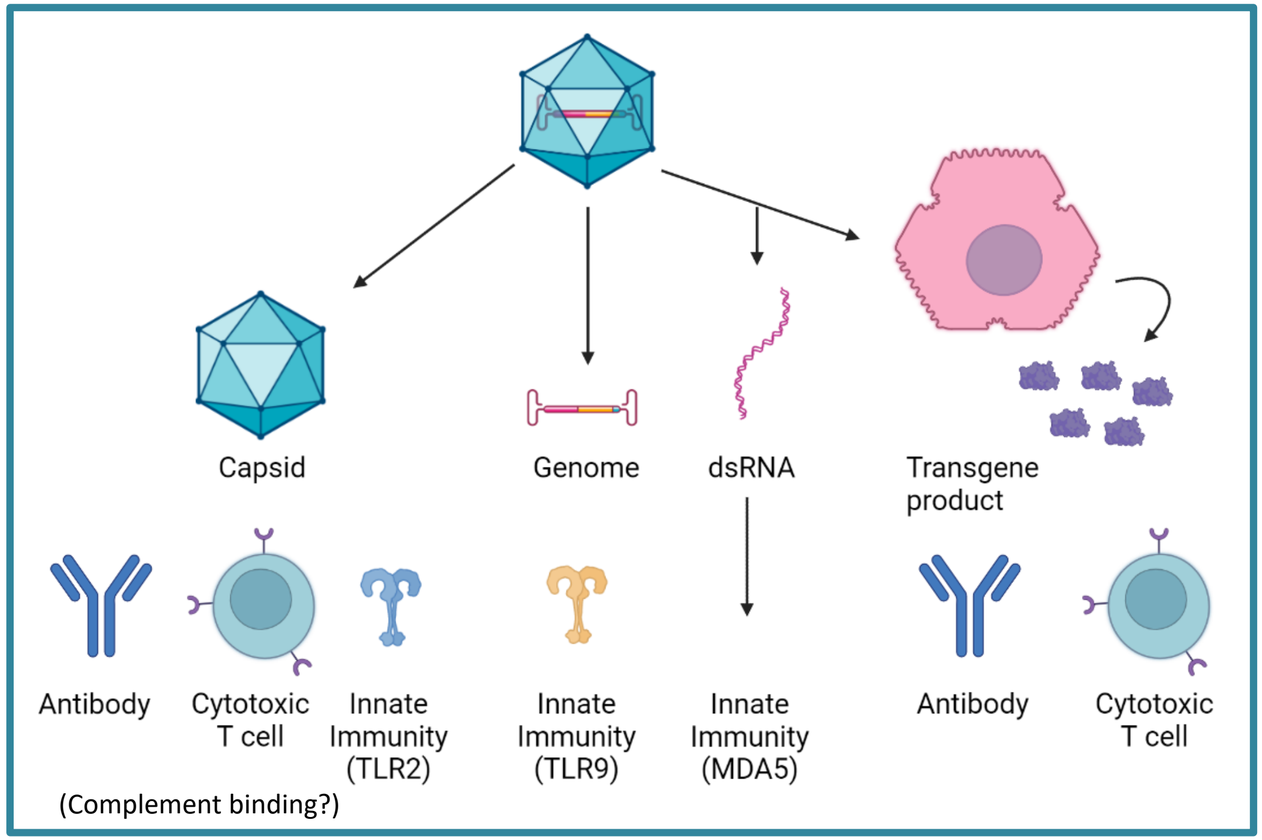
Gene/Genome Editing

- Transient or continued production of editing nucleases
- On-target editing in target cell population
- Off-target editing in target cell population
- Off-target editing in non-target cell population
- Genotoxicity risk assessment (more to follow)

Immunogenicity

- Pre-existing antibody assessment
- Response to AAV vector and/or construct
- Response to expressed transgene

Human immune system and AAV vectors



dsRNA, double-stranded RNA; MDA5, melanoma differentiation-associated protein 5; TLR, Toll-like receptor.
Source: Shirley et al., 2020. Immune Responses to Viral Gene Therapy Vectors. Molecular Therapy 28: 709-722 (16)

Adapted from FDA Cellular, Tissue and Gene Therapy Advisory Committee, Sep 2-3, 2021; using BioRender.com

Pre-existing anti-AAV antibody prevalence is high in older children and adults

- Can block AAV vector activity
- Many clinical trials exclude subjects with pre-existing anti-AAV antibodies
- Companion diagnostic assay

Innate and adaptive immune systems

- Activation of complement pathways
- Anti-AAV T cells can mediate hepatotoxicity

Role for immunosuppressive drugs?

Anti-AAV T cells can mediate hepatotoxicity

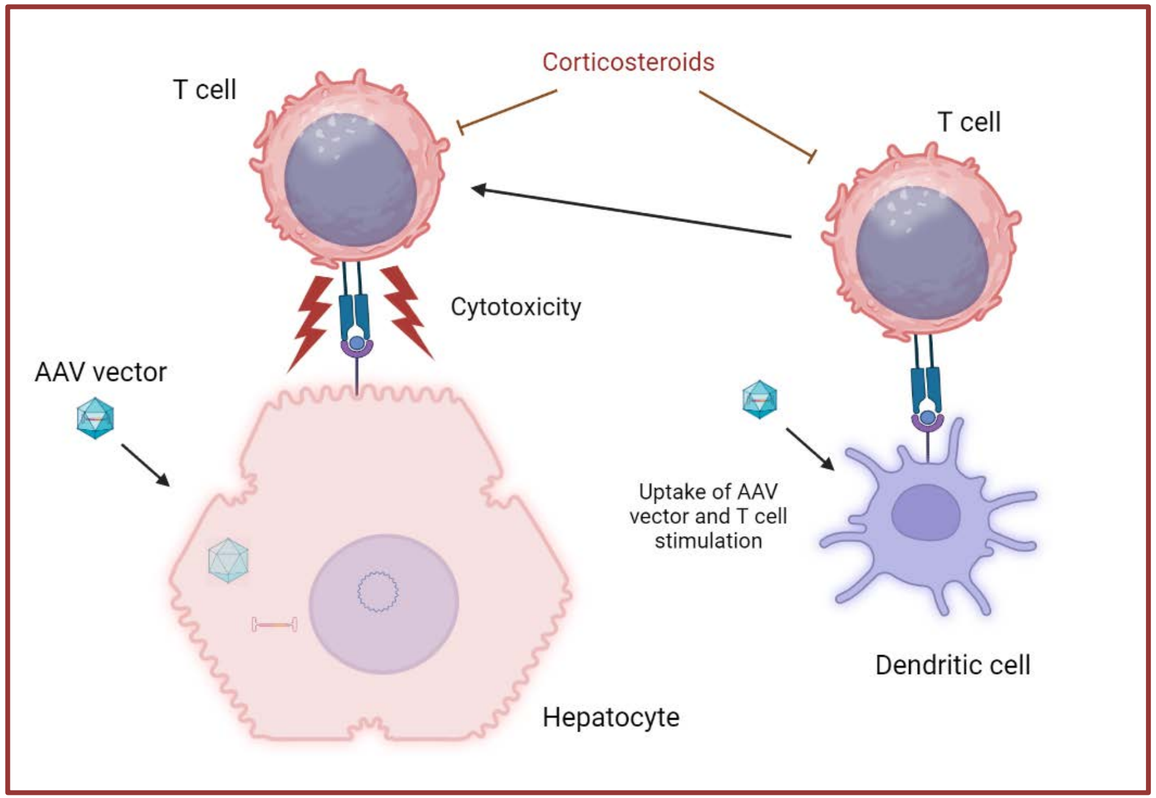


Figure adapted from FDA Cellular, Tissue and Gene Therapy Advisory Committee, Sep 2-3, 2021; created using BioRender.com

T cell response to AAV administration can lead to:

- Increase in ALT and AST levels
- Reduction in transgene expression
- Believed to be related to toxicity of cells expressing transgene
- Immunosuppression has had variable effects in animals and clinical studies
- Not clear-cut as some AAV/transgenes are more problematic than others
- What tips the scale?

Genotoxicity assessment for gene editing

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Assessment	Ex Vivo	In Vivo
Target cells for assessment	Pre-dose modified cells Post-dose cell samples	Biopsy?
Bioinformatics off-target identification	Yes	Yes
Unbiased off-target identification and confirmation	Yes	Yes
Integration evaluation	Yes	Case-by-case
Karyotype	Yes	Not applicable
Molecular translocation analysis	Yes	Yes
In vitro tumorigenicity • Soft agar assay with human fibroblasts for nucleases • IL-2 independent growth for T cell products	 Yes Yes	 Yes No
In vivo (based on robustness/lifespan of edited cell in immunodeficient mice)	Case-by-case	Not applicable
Kinetics of gene editing components expression and activity	Yes	Yes

Genotoxicity risk assessment

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Gene editing off-target analysis

- What is the amount of off-target activity compared to on-target “therapeutic” activity?
- What is the location of off-target site(s) relative to known genes?
  - Are they within an exon? (high risk of impact)
  - Are they within an intron or intergenic? (less risk of impact)
  - Proximity to cancer-associated genes/tumor suppressor genes?
- Consult literature to assess the biological risk of off-target genes

Genotoxicity risk assessment

- Weight-of-evidence
- Risk/benefit considerations

Key safety assessments for GT products (3)

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Traditional Safety Evaluation Endpoints (if feasible)

- Clinical chemistry, hematology, urinalysis, macroscopic and microscopic pathology. EEG, respiratory and ophthalmology endpoints on case-by-case basis
- AAV dorsal root ganglion pathology

Less challenging for In Vivo GT Products (traditional paths)

- Challenges include immunogenicity to foreign human transgene expressed in animal species

More Challenging for Ex Vivo GT Products (product-unique paths)

- Challenges include ability of human cells to proliferate and survive in immunocompromised mouse models
- Many traditional animal efficacy models used for small molecule and/or monoclonal antibody development are not feasible for testing GT products

SAEs in AAV clinical studies

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

Serious adverse events seen in humans were also seen in animal toxicology studies

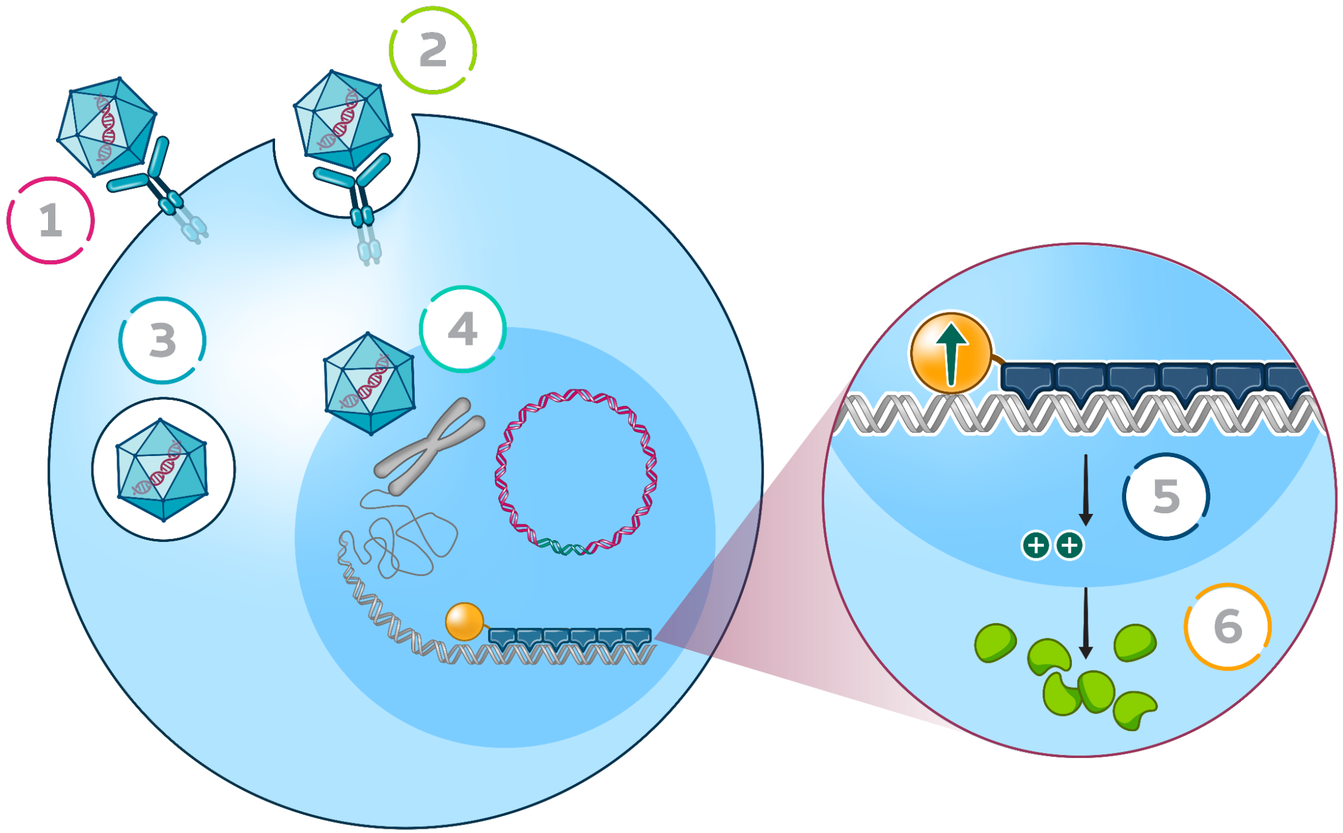
Toxicity	Serious Adverse Events	Vector Serotype	Indication	Route of Administration and Dose	Nonclinical Study Observations
Hepatotoxicity	Elevated liver enzymes, serious liver injury	AAV9	Spinal muscular atrophy (SMA)	Intravenous	Transient elevation in liver enzyme and histopathology (mild/min) findings in neonatal FVB/NJ mice (>1.1e14 vg/kg Zolgensma); Acute liver failure, thrombocytopenia, coagulopathy NHP (2e14 vg/kg AAVhu68); similar findings, along with complement activation, reported in NHPS administered AAV9 or AAV-PHP.eB (1-2e14 vg/kg)
	Elevated liver enzymes	AAV5	Hemophilia	Intravenous	Transient liver enzyme elevation in Hem A dogs and healthy NHPs (up to 4e13 vg/kg in dogs; up to 5e12 vg/kg in NHPs
	Liver failure	AAV8	X-linked myotubular myopathy	Intravenous	No adverse findings in XLMTM mouse (up to 3e13 vg/kg) or dog (up to 5e14 vg/kg) models
Thrombotic microangiopathy (TMA)	Thrombocytopenia, hemolytic anemia, acute kidney injury	AAV9	SMA, Duchenne muscular dystrophy	Intravenous	Acute thrombocytopenia, coagulopathy, transient complement activation and hepatotoxicity in healthy NHPs; no adverse kidney histopathology
Neurotoxicity (DRG Histopathology)	DRG neuronal loss	AAV9	Giant axon neuropathy	Intrathecal/cisterna magna	Degeneration of primary sensory neurons in DRG and axonopathy of spinal cord in NHPs; minimal to moderate in severity with no associated clinical signs; similar findings in mice and Yucatan minipigs
Neurotoxicity (DRG Histopathology)	DRG neuronal loss	AAVrh10	ALS due to SOD1 mutation	Intrathecal	No data
Neurotoxicity (Brain MRI)	Abnormal T2 hyperintensities	AAVrh10	Late infantile Batten disease	Intraparenchymal	Brain MRI and histopathology abnormalities reported in healthy NHPS following intraparenchymal administration, up to 52 weeks; also reported in rats for histopathology findings; no neurobehavioral findings

Adapted from FDA Cellular, Tissue and Gene Therapy Advisory Committee, Sep 2-3, 2021

Summary & Conclusions

GT molecular mechanisms are not fully understood thus extrapolation of animal data to anticipated human response is also not fully understood

HESI IMMUNOSAFETY TECHNICAL COMMITTEE



1. AAV vector with transgene binds to cell receptor
2. AAV internalization and uptake into cell
3. AAV trafficking into cytoplasm
4. AAV trafficking from cytoplasm into nucleus and expression of transgene
5. Specific and selective DNA binding and gene activation
6. Targeted increase in protein levels

Considerations for clinical translation

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

- Consider clinical population and risk/benefit of treatment
- Clinical translation is already challenging for gene therapy programs – adding the genome editing component adds to the complexity
- Characterize the amount of transgene expression needed for desired therapeutic effect in animal disease model(s), pharmacodynamic responses, dose response relationships, and safety profile in animal species
- Understand the genotoxicity risk profile
- Clinical dose selection using case-by-case/weight-of-evidence translation of animal data to anticipated response in humans
- Balance conservative approach for dose selection with need to provide benefit to human subjects in phase 1/2 study

Summary & Conclusions

HESI IMMUNOSAFETY TECHNICAL COMMITTEE

- Gene therapy offers great potential for improving patient health and quality of life
- Gene therapy and genome editing are still new fields and risks not fully characterized
- Genome editing platforms continue to evolve and enhance specificity
- Regulatory guidance on GT and genome editing continues to evolve
- Pharmacologically-relevant animal models are paramount to nonclinical success; especially challenging for human cell products tested in animals.
- Nonclinical studies needed to characterize transduction, biodistribution, transgene expression and kinetics, pharmacodynamics and safety profile
- Nonclinical safety assessment based on tailored case-by-case and weight-of- evidence approach to inform risk/benefit, safety profile and clinical dose selection
- Balance conservative approach for dose selection with need to provide benefit to human subjects in Phase 1/2 study